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Optimizing hatchery practices for genetic improvement of marine bivalves

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Abstract

Aquaculture currently accounts for approximately half of all seafood produced and is the fastest growing farmed food sector globally. Marine bivalve aquaculture, the farming of oysters, mussels and clams, represents a highly sustainable component of this industry and has major potential for global expansion via increased efficiency, and numbers of, production systems. Artificial spat propagation (i.e. settled juveniles) in hatcheries and selective breeding have the potential to offer rapid and widespread gains for molluscan aquaculture industry. However, bivalves have unique life-histories, genetic and genomic characteristics, which present significant challenges to achieving such genetic improvement. Selection pressures experienced by bivalve larvae and spat in the wild contribute to drive population structure and animal fitness. Similarly, domestication selection is likely to act on hatchery-produced spat, the full implications of which have not been fully explored. In this review, we outline the key features of these taxa and production practices applied in bivalve aquaculture, which have the potential to affect the genetic and phenotypic variability of hatchery-propagated stock. Alongside, we compare artificial and natural processes experienced by bivalves to investigate the possible consequences of hatchery propagation on stock production. In addition, we identify key areas of investigation that need to be prioritized to continue to the advancement of bivalve genetic improvement via selective breeding. The growing accessibility of next-generation sequencing technology and high-powered computational capabilities facilitate the implementation of novel genomic tools in breeding programmes of aquatic species. These emerging techniques represent an exciting opportunity for sustainably expanding the bivalve aquaculture sector.

Key words: gene-environment interactions, genomic selection, marine bivalve aquaculture, selective breeding, sustainable development.

The future of bivalve aquaculture relies on artificial propagation

With the global human population projected to exceed 9 billion by 2050, food production must increase by at least 59% to meet projected demand (Valin *et al.* 2014). Feeding this growing population, whilst maintaining biodiversity and good environmental stewardship, is one of the major global challenges of the 21st century. This issue is exacerbated further by the need to ensure that the future intensification of food production is sustainable, especially in the face of climate change (UN 2015; IPCC 2018).

Aquaculture is the fastest growing food production sector globally, expanding on average 6.4% per annum since 2001 (Subasinghe 2017). Nearly half of the current global finfish and shellfish production derives from aquaculture (FAO 2019a), with this sector expected to underpin most future growth in seafood production (Kobayashi *et al.* 2015). Currently, mollusc farming accounts for approximately 21 % of world aquaculture production (Subasinghe 2017).

Important scientific advances in bivalve husbandry practices (i.e. optimization of diet, fertilization protocols and larval rearing) occurring within the last century (e.g. Galstoff 1938; Carriker 1956; Loosanoff & Davis 1963) led to the establishment of the first commercial bivalve mollusc hatcheries (Mann 1983) resulting in the global expansion of shellfish aquaculture. The ability to control environmental conditions in indoor facilities enables broodstock conditioning and spat production almost year-round. Most importantly, the development of a constant and reliable source of spat benefits the expansion of the bivalve aquaculture sector, facilitating the predictability of production and enabling the possibility of selective breeding.

Hatcheries are expected to play a key role in the continued expansion of bivalve aquaculture. The potential of hatchery production is highlighted in China, which accounts for 80% of global production of Pacific oysters (*Crassostrea* gigas) (Yang *et al.* 2014), and the sector now relies almost entirely on hatchery sourced spat (Li *et al.* 2011). Nonetheless, demand for hatchery-produced spat is often low in areas where natural (wild capture) spat is available and abundant. A similar situation occurs in France, which is responsible for 82% of Pacific oyster production in Europe (92 000 tonnes in 2018 (Eurostat 2020)) where over 60% of spat is captured from wild sources (Richez 2012). This contributes to a slow shift from a natural to hatchery production model (Adamson *et al.* 2017). This same production template is also true for mussels; currently, industries for two of the main farmed species, the blue mussel (genus Mytilus) in Europe (Kamermans *et al.* 2013) and the green-lipped mussel (Perna canaliculus) in New Zealand (Symonds *et al.* 2019) still rely primarily on natural spat. This process is an inexpensive but unreliable practice, which is vulnerable to habitat disturbances and restricts the development of cultivation technologies such as selective breeding.

The Food and Agriculture Organization (FAO) has recently proposed a number of key developments, which will assist the aquaculture industry in addressing several long-term sustainability challenges (FAO 2016). One priority area highlighted by the FAO is the use of stock management and selective breeding to produce lines with greater reliability and productivity in a wide range of environments (FAO 2019b). To date, encouraging responses to selection have been observed in aquatic species: the average gain in body weight per generation is 8.7% in shrimps, 10.3% in oysters and between 9% and 17.9% among finfish species (Gjedrem & Rye 2018). Although recent estimates show that in the 10 main farmed aquatic species 75% of production benefits from some form of selection (Houston *et al.* 2020), only a small percentage of global aquaculture production (<10% in 2012) utilizes genetically improved stock (Gjedrem *et al.* 2012).

Despite an increasing availability of genomic resources for bivalves, the mechanisms underlying domestication (i.e. adaptation to a farmed environment), and genotype-environment interactions (*GxE*) ongoing in cultured bivalve species remain poorly studied. The degree to which these processes influence the response to selection in these taxa, and consequently the potential to genetically improve organisms, represent two key knowledge gaps with respect to bivalve selective breeding (Figs 1 and 2). Accordingly, the potential for losses in genetic diversity during production is exacerbated and likely hinders the efficiency of existing hatchery management and selective breeding programmes, jeopardizing sustainable growth of this sector. There is a fundamental need to clarify the impacts of hatchery-management practices on the genetic and phenotypic constitution of cohorts, and the resulting long-term implications for bivalve production.

In this review, we explore the mechanisms by which production practices and life-history characteristics can influence the genetic variability and quality of spat during hatchery-propagation (Fig. 1). We describe the current status of selection in bivalve aquaculture globally and the different methods employed for production. Further, we discuss how management practices potentially benefit or hinder the optimization of selective breeding approaches, and how a greater control of hatchery-propagation processes can contribute to the sustainable intensification of bivalve aquaculture. There are considerably fewer studies investigating the consequences of domestication selection in bivalves in comparison with other aquatic species. Therefore, in order to infer the possible consequences of artificial propagation in these organisms, we compare the selection pressures acting in hatcheries with those acting in the wild, when applicable. By identifying the main gaps in knowledge and bringing awareness to this topic, we expect to contribute to increasing efficiency and accuracy of selection in these taxa and inspire future research which may contribute to increasing efficiency and accuracy of selection in marine bivalves.

Selective breeding in bivalve aquaculture: current status and opportunities

Successful breeding programmes have been established for bivalves worldwide and include those applying mass and family selection approaches (Table 1) (Hollenbeck & Johnston 2018). In mass selection, individuals are typically selected according to their performance in comparison to the population's mean for a specific trait (e.g. growth) without fully accounting for family structure. This strategy can be effective but runs the risk of inbreeding depression and is only suitable for a focus on one or two traits. Alternatively, family selection is based on pedigree information,

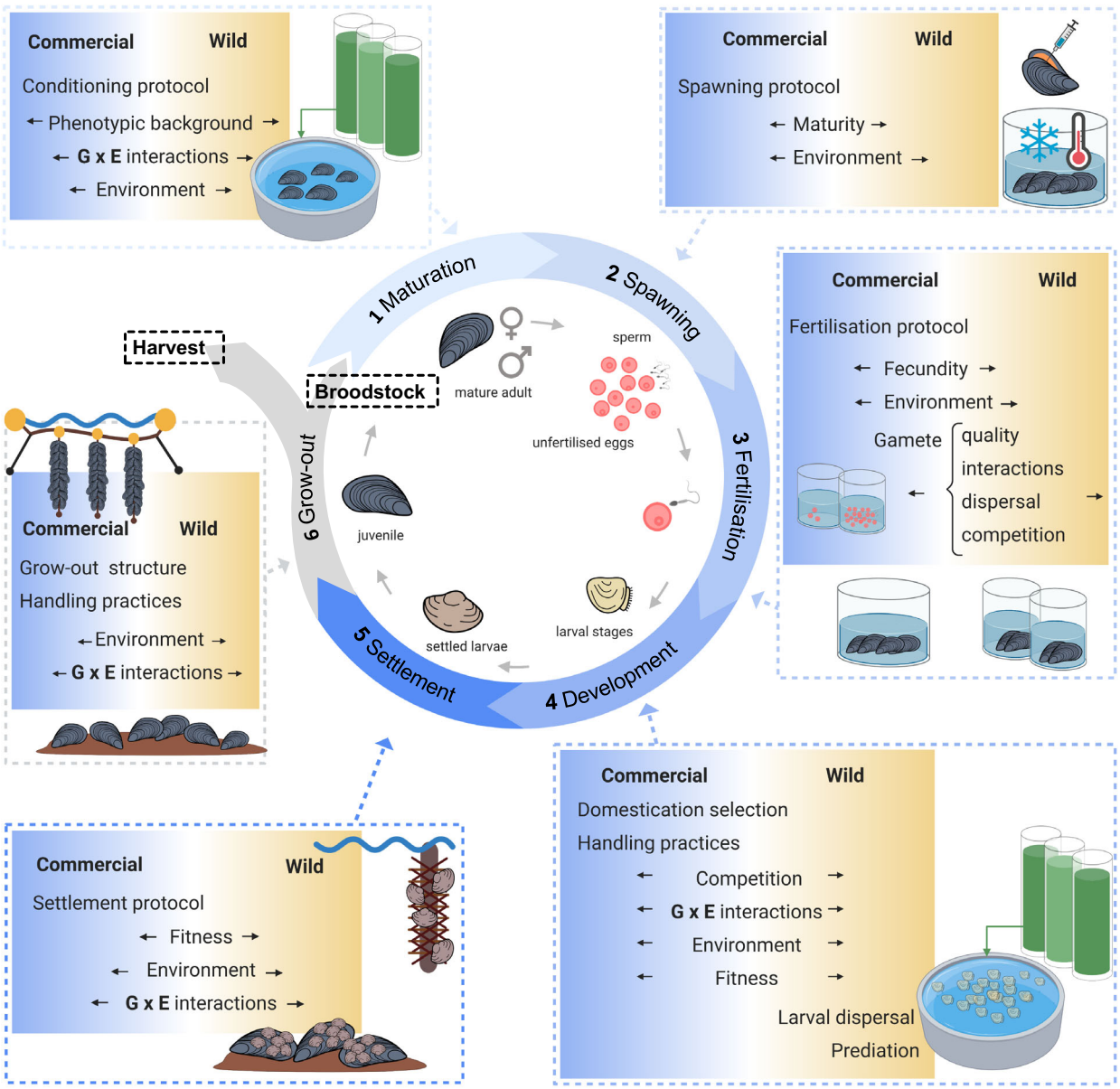

Figure 1 Concept diagram highlighting the selection pressures acting upon natural and artificially propagated bivalve stocks, across different life-cycle stages and corresponding production steps. Selection pressures (coloured boxes) acting upon wild populations (indicated with yellow background), hatchery populations (indicated with blue background) or both (indicated with flanking arrows), as generated by the artificial or natural environment. Selection pressures correspond to each production step discussed within the review (steps 1 - 5; maturation, spawning, fertilization, development, settlement and grow-out; outer circle), as well as the corresponding life-cycle stage (inner circle). Figure created with BioRender.com.

and individuals from the top performing families are chosen to form the breeding populations, allowing for effective maintenance of genetic diversity. Family-based selection has been applied in a commercial *M. galloprovinciallis* breeding programme. Here, the use of 77 full-sib families resulted in a heritably of 0.35 (SE = 0.09) for total weight and 0.23 (SE = 0.08) for meat yield as a ratio between meat weight and total weight, after 2 generations; both of which are commercially relevant traits (Nguyen *et al.* 2014).

Selection has also successfully improved traits such as growth rate (Hershberger *et al.* 1984; de Melo *et al.* 2016), disease resistance (Naciri-Graven *et al.* 1998; Dove *et al.*

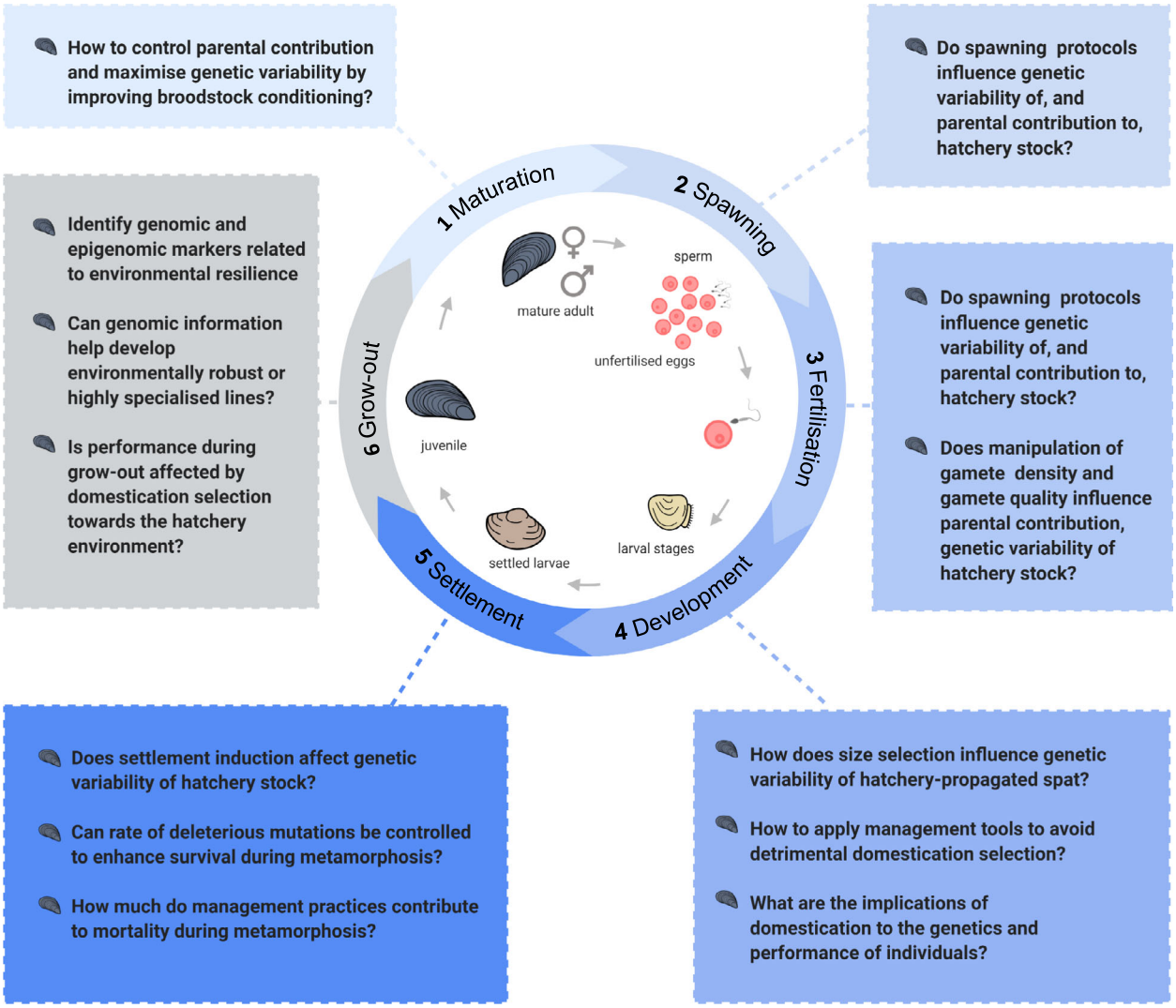

Figure 2 Critical knowledge gaps currently preventing the widespread implementation of genomic breeding approaches in bivalve aquaculture. Main knowledge gaps as identified in this review and considered as key priorities for future research (coloured boxes). Knowledge gaps are linked to each production step discussed in this review (steps 1 - 5; maturation, spawning, fertilization, development, settlement and grow-out; outer circle) as well as the corresponding life-cycle stage (inner circle). Figure created with BioRender.com.

2013a; Dégremont *et al.* 2015b) and resilience towards environmental perturbation (Parker *et al.* 2015). Despite these success stories, bivalve aquaculture production still relies greatly on wild type strains (Hollenbeck & Johnston 2018), that may not be adapted to the farming environment (Yáñez *et al.* 2015). Therefore, significant potential for genomic improvement exists, providing the opportunity to maximize productivity for bivalve aquaculture species worldwide.

The recent development and increasing affordability of high-throughput sequencing technologies have facilitated the incorporation of genomic tools in breeding programmes of aquatic species (Zenger *et al.* 2019). This has enabled a step forward from family selection, particularly for traits which are difficult or impossible to measure directly on selection candidates, such as disease resistance. For such traits, family selection would only allow for family level breeding values, thereby missing out on within-family genetic variation. Genomic tools allow breeders to access and utilize the within-family component of genetic variation. This can be achieved in two main ways. Firstly, mapping of quantitative trait loci (QTL) allows the identification of genetic markers significantly associated with a specific trait within the species of interest. Selection for traits with large effect QTLs can be improved by applying marker-assisted selection (Zenger *et al.* 2019). Secondly,

Table 1 Large-scale breeding programmes for cultured marine bivalve species (adapted from Hollenbeck & Johnston, 2018)

Common name	Species	Group	Location	Type of selection	Programme type	Founded	References
Mediterranean mussel	*Mytilus galloprovincialis*	Mussel	Australia	Family	Industrial	2008	Nguyen and Ingram (2012)
Greenlip mussel	*Perna canaliculus*	Mussel	New Zealand	Family	Industrial	1999	Camara and Symonds (2014)
Pacific oyster	*Crassostrea gigas*	Oyster	USA	Family	Industrial	1996	de Melo *et al*. (2016), Langdon *et al*. (2003)
Pacific oyster	*Crassostrea gigas*	Oyster	Australia	Family; mass	Industrial	1997	Kube *et al*. (2011), Ward *et al*. (2005)
Pacific oyster	*Crassostrea gigas*	Oyster	New Zealand	Family	Industrial	1999	Camara and Symonds (2014)
Pacific oyster	*Crassostrea gigas*	Oyster	France	Mass	Experimental	2009	Dégremont *et al*. (2015b)
Pacific oyster	*Crassostrea gigas*	Oyster	China	Mass	Experimental	2007	Li *et al*. (2011), Zhong *et al*. (2016)
Sydney rock oyster	*Saccostrea glomerata*	Oyster	Australia	Family; mass	Industrial	1990	Dove *et al*. (2013b), Nell *et al*. (1996), Nell *et al*. (1999)
Bay Scallop	*Argopecten irradians*	Scallop	China	Mass	Unknown	2001	Zheng *et al*. (2004), Zheng *et al*. (2006)

genomic selection can be applied for selection of polygenic traits (Meuwissen *et al*. 2016). Such approach can cover a large number of loci across the genome and provides enough information to capture all causative variants for a given trait, as loci are expected to be in linkage disequilibrium with one or more common markers (Meuwissen *et al*. 2001). Besides, genomic selection captures the within-family variance as markers shared between individuals can be identified, increasing the accuracy of the estimated breeding values and response to selection (see Zenger *et al*. 2019 and references therein). Additionally, it enables retrospective parental assignment, which allows multiple families to be grown in mixed tanks and reduces the generation of confounding genetic and environmental effects. Genomic selection can also be designed to fit different levels of ploidy (Ashraf *et al*. 2016; Endelman *et al*. 2018) and can be a valuable asset to guarantee a high precision in breeding programmes utilizing broodstock with increased value through ploidy manipulation.

Having a set of tools which link high-resolution genetics with phenotypes is a main requirement for genomic selection. To date, genomes have been assembled for several of the main cultured species (Hollenbeck & Johnston 2018). In addition, the development of DNA markers including microsatellites (Li *et al*. 2003; Wang *et al*. 2016) and single nucleotide polymorphisms (SNPs) (Sauvage *et al*. 2007; Fleury *et al*. 2009; Nguyen *et al*. 2014; Wang *et al*. 2015; Vu *et al*. 2021) as well as the identification of genomic regions associated to traits of economic importance through QTL mapping (Sauvage *et al*. 2010; Guo *et al*. 2012; Jiao *et al*. 2014) and genome-wide association studies (GWAS) (Gutierrez *et al*. 2018; Meng *et al*. 2019), create a genomic toolbox which provides a backbone for future research. Ultimately, this information promotes the development of genomic based selection techniques and the fine-tuning of breeding programmes.

Marine bivalves share complex genomic and life-history features, including high levels of nuclear genetic diversity, high heterozygosity, and elevated numbers of deleterious mutations and null alleles (Bierne *et al*. 1998; Plough & Hedgecock 2011; Hollenbeck & Johnston 2018; Gerdol *et al*., 2019). In addition, reproductive attributes (broadcast spawning, high fecundity, high early mortality rates), and a high variance in reproductive success (Vk) among individuals (Hedgecock & Pudovkin 2011), are commonly described in these taxa. Variance in reproductive success can result in low effective population sizes (Ne) and low numbers of effective breeders (Nb) relative to census size, termed 'sweepstake reproduction', which has been observed in both wild and hatchery-propagated stock (Hedgecock & Sly 1990; Hedgecock 1994; Boudry *et al*. 2002; Plough & Hedgecock 2011). Heterozygous deficiencies relative to Hardy-Weinberg equilibrium, and segregation distortion of markers described in paired crosses, are also commonly reported in bivalves (Launey & Hedgecock 2001; Peñaloza *et al*. 2014).

These properties of the bivalve genome, together with specific life-history characteristics of these organisms may influence the efficiency and applicability of genomic resources in breeding programmes. Therefore, efforts to elucidate the role these features play in the selection process are vital to enhance production in this sector. Selection must focus on traits that enhance larval performance and productivity, whilst simultaneously selecting for traits which are relevant in later development. Thus, another key priority is to understand the genetic basis of these traits, as well as their genetic and developmental correlations.

Genomic resources have the potential to revolutionize aquaculture production, contributing to the rapid expansion and optimization of marine bivalve production. Nonetheless, socioeconomic factors also play a key role in the implementation of new technologies in existing production systems, and may slow down the pace of genomic breeding in aquaculture, especially in developing countries (Kumar *et al.* 2018). To date, industrial applications of genomic selection in aquatic species are limited, and largely restricted to finfish species (Zenger *et al.* 2019).

Broodstock conditioning and its implications on genetic variability

Contrary to natural ecosystems, hatcheries offer a largely uniform environment to cultivate broodstock, reducing sources of stress caused by sub-optimal or fluctuating, conditions. In these artificial systems environmental conditions can be manipulated to trigger gametogenesis in broodstock throughout the year, extending the period through which mature breeders are available (Helm 2004). Overall, the process of induced gametogenesis, known as conditioning, aims to maximize the fecundity of progenitors whilst maintaining the high quality of gametes and larval viability (Lannan *et al.* 1980; Utting & Millican 1997). For aquaculture purposes, broodstock are either collected in their natural environment or taken from previous generations of hatchery stock and are held in flow-through systems (Helm 2004). During the conditioning process, quality and availability of food resources have a direct effect on adult fecundity levels and reproductive output (Utting & Millican 1997), with lipid and proteins obtained from food accumulated during oogenesis (Li *et al.* 2000). A significant correlation between biochemical content of oocytes and early developmental success (Massapina *et al.* 1999; Corporeau *et al.* 2012; Boulais *et al.* 2015), highlights the vital role that conditioning can play in production, and consequently, in the genetic makeup of cohorts (Fig. 1).

To date, standard conditioning protocols have been established for the main cultured bivalve species (Helm 2004). However, a large (up to twofold) variation in length of conditioning period is reported among strategies adopted by different hatcheries, and a quality check of broodstock gonad development is not consistently undertaken among hatcheries (de Reynaga-Franco *et al.* 2020). Without equal opportunity for success in breeding, Nb/N ratio is lowered. In addition, an unsynchronized response of broodstock to conditioning may reduce the potential number of breeding pairs, promote discrepancies of both Vk among individuals and performance among families (Boudry *et al.* 2002), with inbreeding levels within a breeding programme consequently increasing. Such issues rapidly nullify predictive ability of selective breeding methods and impose a challenge for the implementation of genomic selection in these taxa.

Genomic and phenotypic consequences of hatchery propagation

In hatcheries, spawning of broodstock can be triggered either by non-lethal techniques (thermal cycling, intermittent exposure to air and/or introduction of potassium chloride, hydrogen peroxide, steroids or neurotransmitters in the mantle cavity or adductor muscle) or by stripping (scarifying) the gonads of individuals (Helm 2004). The adoption of gonad-stripping or chemically induced spawning protocols can help to standardize the time of gamete release, reducing the deterioration of gametes. However, such approaches do not discriminate between mature and immature gametes present in the gonad. The lack of control of gamete quality during artificial spawning may lead to a high variability in developmental rate within a batch (Tanyaros & Tarangkoon 2016). In fact, for some species such as *M. edulis*, gonad stripping is a non-viable approach which impairs production (Kamermans *et al.* 2013). Moreover, the required sacrifice of pedigreed broodstock individuals (where identified) may render this approach unfavourable for selective breeding.

Owing to its practicality, mass spawning (combining gametes from multiple females with an aliquot of pooled male gametes) is a common procedure for artificial fertilization (Helm 2004; Tetrault 2012). This approach does not control parental contribution and can result in reduced numbers of effective parents in the programme. Moreover, as best performing individuals may be excluded from crosses, mass selection can limit the accuracy of the breeding programme. However, fecundity levels observed in bivalves are high and fertilization is commonly successful, and a sufficient number of offspring is often achieved. Inbreeding load, as well as impaired development, can be concealed by management practices (e.g. culling) where the low performing individuals are eliminated from a batch by size selection (Taris *et al.* 2006). As genomic and marker assisted selection endeavour to capture favourable genetic variation, it is vital to identify and control for the possible impacts of spawning and fertilization protocols on the genetic variability and performance of cohorts (Fig. 2).

To overcome issues with parental contribution, pairs can be individually crossed. Paired crossing is less commonly adopted in hatcheries as it is a more laborious approach, requiring the control of fertilization rates of individual crosses and investment in personnel and equipment. This method demands additional physical space to separate mating pairs and subsequent offspring during larval development. Furthermore, the rearing of juveniles in family-specific tanks presents an issue with confounding of genetic

and common environmental effects, which would require multiple replicate tanks per family to resolve. Subsequently mixing families, and growing them together in a common environment, can mitigate against this issue. However, the gamete density used in artificial crosses is substantially higher than in nature. Empirical evidence demonstrates that mass spawning increases V_k among males and pair crossing individuals increases the variance in reproductive success among females (Hornick & Plough 2019). Handling practices may additionally contribute to increase variation in family sizes, which often goes undetected. Long term, such practices can bottleneck genetic variability in artificially propagated stock and dramatically reduces the ability to predict success for selective breeding. Therefore, altered genetic diversity of hatchery-propagated stock is, an inevitable consequence of the chosen fertilization approach (Fig. 1) (Hornick & Plough 2019).

Phenomena occurring at the gamete level may also play a role in determining parental contribution in crosses and act as an early selective pressure. For example, the distance which sperm must travel to reach oocytes, gamete phenotype (biochemical composition, sperm motility and behaviour, oocyte size and age) and gamete interactions influence the success of fertilization (Levitan 2006; Suquet *et al.* 2010; Boulais *et al.* 2015; Boulais *et al.* 2017). Genetic compatibility can influence fertilization success, favouring crosses between less related individuals (Lymbery *et al.* 2017). In sea urchins, low sperm densities favoured crosses between common genotypes which match at the gamete binding locus (oocyte-sperm compatibility locus) (Levitan & Ferrell 2006). Sperm-saturation, in turn, promoted reproductive success of individuals with less frequent genotypes. These findings highlight the putative role of gamete density in sperm choice behaviour. Factors such as affinity between crosses (Kekäläinen & Evans 2017) and sperm longevity (Crean *et al.* 2012) have been linked to increased postzygotic fitness. However, the extent to which interactions at the gamete-level, as well as gamete phenotype, influences fertilization success in external fertilization is not yet fully understood (Breed & Moore 2015).

The precise determination of oocyte-sperm ratios and controlled-crossing approaches may benefit fertilization success and enhance the contribution of individual broodstock (Song *et al.* 2009). Gamete density used in artificial crosses is substantially higher than in nature, increasing competition among individuals, and acts on Nb. Oocyte mechanisms acting against polyspermy are not 100% effective, thus, increased competition can lower the rates of fertilization success among crosses. Commonly, substantial variation in gamete phenotype and fertilization rates are commonly observed among and within individuals (Breed & Moore 2015). During hatchery propagation, gamete quality is assessed via crude visual observations of sperm motility and concentration, as well as shape (roundness), size and colouration of oocytes. Individuals classified with high quality gametes are selected for fertilization, whilst those not meeting the quality criteria are excluded from crosses. Correlations between gamete phenotypes (e.g. oocyte biochemical composition) and larval viability in artificially bred bivalves and other invertebrates have been previously described (Massapina *et al.* 1999; Crean *et al.* 2012; Boulais *et al.* 2015). Nonetheless, the extent to which gamete traits and gamete-level interactions influence V_k and genetic variability of offspring is not fully understood. If such implications can be carried over throughout the individuals' life, the expression of key genotypes may be modulated by pre-fertilization selection. However, further investigation is required to clarify how physiological and molecular mechanisms underlie gamete phenotype, and affinity of crosses during external fertilization (Fig. 2). Such knowledge can benefit the development of mating systems that maximize fertilization and homogenize V_k among breeders.

Hatchery-propagated larvae are reared in a controlled environment, avoiding the risks imposed by oceanic drift and predation. In this environment, water quality parameters are maintained at, or close to, conditions considered optimal for the survival of the species being cultured. This optimized environment enables the levels of production to be improved, maximizing larval growth and settlement rates of the produced species. In the long term, domestication contributes to enhance performance under these artificial rearing conditions. However, domestication selection can lower environmental resilience when exposed to natural conditions. A lower fitness of individuals in the wild has been observed in fish species which are currently in transition to a domesticated status (Araki *et al.* 2008). Moreover, genomic footprints of domestication can vary greatly between populations from independent origins selected for the same trait (López *et al.* 2019), as a result of the specific characteristics of a rearing environment (Vandeputte *et al.* 2009). Recent findings indicate that selectively bred *C. virginica* larvae were less able to tolerate starvation compared with wild cohorts, experiencing significantly higher mortality rates (McFarland *et al.* 2020). However, the genomic mechanisms underlying domestication selection of marine bivalve species remains poorly investigated in comparison to finfish species. Optimization of selective breeding will require these factors to be better understood and controlled for.

Culling, or size selection, is commonly practised in hatcheries throughout larval development. Selection for similar growth rates under culture conditions generally improves overall spat production and reduces variation in development within a cohort (Taris *et al.* 2006), but may potentially mask the signs of inbreeding depression (Taris *et al.* 2007). Therefore, such a practice may benefit early

production stages. However, the effect of size selection on a stocks' genetic variation are not clear (Fig. 2). Culling may result in accidental removal of individuals that may reach market size quickest in later development, individuals with alternative traits of interest (e.g. disease resistance) or traits that are relevant during later stages of production (e.g. robustness), directly impacting a breeding scheme. A practical example is seen in *Mercenaria mercenaria* larvae, where initially small individuals present in culture tanks are capable of surpassing the size of individuals that were initially larger, at later stages of development (Gionet *et al.* 2010). In addition, this process can reduce genetic variability of offspring (Taris *et al.* 2006) acting as a genetic bottleneck in hatcheries. Losses of entire cohorts can result from sudden shifts in conditions when *G*x*E* interactions mean that the animals selected as optimal under a hatchery production environment perform poorly in a subsequent grow-out environment.

Developmental plasticity (input during early development persisting in adult phenotype) can modify the performance of individuals in their later life. For example, exposure of quagga mussel larvae (*Dreissena bugensis)* to a range of temperatures has been correlated with the development of different shell morphotypes in adults (Peyer *et al.* 2010). If early exposure to stressors can imprint performance of organisms in later life, alternative culling strategies (e.g. application of a salinity or temperature shock during early development) would enable selection for robustness to future environmental conditions. Accordingly, the hatchery environment and management practices may themselves help or hinder spat development, potentially affecting the performance of individuals at grow-out sites (Reynaga-Franco *et al.* 2019).

Currently, research into the implications of hatchery practices on the genetic characteristics of bivalves is restricted to a few studies (Boudry *et al.* 2002; Taris *et al.* 2006; Taris *et al.* 2007; Lallias *et al.* 2010; Hornick & Plough 2019; McFarland *et al.* 2020). Unravelling the genomic basis of environmental resilience will allow the potential of selection towards robustness, or generalist phenotypes, and its association with other commercially relevant QTL to be determined (Vu *et al.* 2021). Additionally, the development of physiological indices of larval performance, and their association with the individual genotype, can contribute to improve selection in these taxa (Pan *et al.* 2016). Further studies clarifying the correlation between larval performance of hatchery selected stock and juvenile and adult performance during grow-out will contribute to the development of breeding strategies and optimization of production throughout the entire life cycle of these taxa.

Hatchery bred spat, which have reached the settlement stage, are often induced to settle. This practice not only facilitates efficient husbandry but avoids any adverse consequences (e.g. depleted energy reserves) of spending too long in the pediveliger stage. Uniformity in settlement time can be achieved by manipulating environmental stimuli such as temperature shocks, or via the addition of fine shell particles or other material to induce settlement in tanks (Helm 2004). Alternatively, settlement of larvae can be chemically induced by exposure to neurotransmitters (Sánchez-Lazo & Martínez-Pita 2012; Grant *et al.* 2013; Joyce & Vogeler 2018). Further investigation is needed to elucidate the role such approaches play as a selective pressure in the hatchery environment and whether these can be used to select or induce favourable characteristics (Fig. 2).

Settlement and metamorphosis are critical moments in the life cycle of bivalves. Substantial mortalities occur during these stages in both natural populations and artificially propagated stock (Hunt & Scheibling 1997; Plough & Hedgecock 2011; Plough 2016), with survival at the post-settlement stage reaching only 2.8% of the original population in some cases (Plough 2016). Genotype-dependent mortality linked to deleterious recessive mutations can occur immediately before or during metamorphosis (Plough & Hedgecock 2011; Plough 2016). Insights on genotype-dependent mortality during settlement have opened the opportunity to investigating the applicability of QTLs to select for uniformity of settlement timing (Plough 2016). In contrast, mortality in the period immediately post-settlement is lower, with no indication of being genotype-dependent (Dégremont *et al.* 2007; Plough 2016).

Genotype by environment responses to the grow-out environment

All spat, both wild and hatchery propagated, are exposed to environmental variability experienced within the coastal and estuarine zones in which grow-out occurs, and are thus susceptible to this daily and seasonal variability (Fig. 1). To thrive in such demanding environments, individuals must either be genetically adapted to extreme conditions, or possess highly plastic physiological responses which allow them to regulate internal mechanisms.

Accordingly, Pacific oysters have demonstrated the ability to regulate genes involved in stress response pathways when facing abiotic stress conditions, including elevated temperature and air exposure (Zhang *et al.* 2012). These findings suggest that a high level of plasticity is a strategy which has allowed these sessile organisms to successfully colonize stressful environments. The expansion of gene families that function as part of the organism's response against biotic and abiotic stress, as well as immune response, suggest that this group has adapted to a sessile life in fluctuating environments (i.e. intertidal coastal and estuarine waters). A better understanding of plasticity mechanisms in bivalves can contribute to the development of

culturing conditions which improve performance in desirable traits. The selection processes experienced during early development in hatcheries could contribute to direct effects on the performance of cohorts, as well as increasing the likelihood of stochastic *GxE* interactions.

GxE is an important factor dictating performance of aquaculture species (see review by Sae-Lim *et al.* 2016 and references therein). Where animals from a breeding programme are reared in different environments, it can result in a re-ranking of families or genotypes. This can negatively impact genetic gain and the effectiveness of a breeding programme. These effects have been observed in previous studies investigating the variation in *C. gigas* performance among families across grow-out sites (Langdon *et al.* 2003; Evans & Langdon 2006). In other cases, selected genotypes outperform in certain conditions, but become poor performers when exposed to a different set of conditions – that is re-ranking of genotypes (Langdon *et al.* 2003; Dégremont *et al.* 2005; Evans & Langdon 2006; Wang *et al.* 2013). In marine bivalves, between-family variance described for traits such as growth, survival and environmental resilience (Dégremont *et al.* 2005; Dégremont *et al.* 2015a; Scanes *et al.* 2020) indicates the genetic basis of traits associated with performance (Vu *et al.* 2021). The re-ranking of genotypes, in turn, highlights the intrinsic effect of *GxE* on overall performance of a family or cohort. There is also genetic variation in how well animals perform across diverse environmental conditions, and this robustness of genotypes to diverse conditions can be analysed using reaction norms, and potentially incorporated into breeding goals to help tackle the impact of *GxE* (Hill & Mulder 2010).

Epigenetic mechanisms (e.g. DNA methylation, histone modifications, non-coding RNAs) are a relevant component of *GxE* interactions, through exposure mediated *GxE*. These mechanisms can modify a phenotype without changing the DNA sequence and can have long-lasting effects (Jablonka & Lamb 2002). In the last decades, new technologies have facilitated the study of epigenetics, providing insights into the contribution of the epigenome to the expressed phenotypes in response to the environment. Among the wide scale of techniques available to study epigenetic regulation, DNA methylation has received the most attention in marine bivalves. In *C. gigas*, DNA methylation patterns have been associated with gene function (Gavery & Roberts 2010) and have been linked to gene regulation (Riviere *et al.* 2013; Olson & Roberts 2014). Environmental heterogeneity has been associated with divergent DNA methylation patterns among *C. virginica* populations (Johnson & Kelly 2020). In *Mytilus galloprovincialis* and the New Zealand pygmy mussel *Xenostrobus secures*, methylation patterns of invasive populations differ from populations in their native range (Ardura *et al.* 2018). Whilst epigenetics can contribute to rapid and transient plasticity in response to stress and environment in marine bivalves, future studies combining genomic and epigenomic information are needed to elucidate the processes underlying *GxE* interactions and phenotype expression in these taxa.

Recent evidence also underlines the adaptive nature of phenotypic plasticity in traits involved in environmental resilience (Li *et al.* 2018). Domestication, or the reduction in environmental variation in early life stages, is unlikely to select for plasticity and may lead to epigenetic profiles that are less suited to the farm environment. However, the impact of artificial-breeding and early life hatchery condition on the epigenome of marine bivalve species remains unresolved. Selective pressures acting during hatchery-propagation most likely favour domestication rather than adaptation towards variable natural environments. Therefore, the potential of hatchery-propagated stock to cope with environmental stress may be reduced during breeding and hatchery processes. Indeed, the epigenome of artificially bred Atlantic salmon differs greatly from wild populations, and the reduced fitness of hatchery-propagated stock in comparison to wild populations is likely a consequence of such variation (Le Luyer *et al.* 2017). Accordingly, signs of lower tolerance to environmental stress in *C. virignica* have been linked to domestication selection (McFarland *et al.* 2020). Here, we emphasize that domestication selection in early life stages could result in high discrepancies in performance and lower the mean performance of cohorts through *GxE* interactions.

For a single species, the commercial market may expand across multiple production environments, which may have to be reflected in the data collection for a breeding programme. In a simple breeding programme design, the breeding candidates and the test animals are the same and held in one environment. In a more advanced design, the selection decisions are also based on the performance records of pedigreed full- and half-sibs of the candidates held at test stations (sib-testing design). The breeding candidates are normally reared at a single breeding nucleus farm, where strict biosecurity and sanitary restrictions are imposed to prevent serious pathogens from entering the breeding nucleus. However, the breeding candidates may as well be reared at a few locations from which the families are produced and at a later and safer stage are transported to the central breeding nucleus. In both cases, this structure may induce GxE effects which may have to be accounted for.

To understand the role of *GxE* interactions in the expression of phenotypes, the performance of different lines needs to be tested in a range of environments. Strong *GxE* interactions could be countered by the creation of specific breeding programmes targeting specific grow-out environments (Dégremont *et al.* 2007). However, it would first be wise to understand if any of the potential hatchery stressors or selection events described herein are contributors to *GxE*

events, and if they can be mitigated through alteration in early life selection. As a crude hypothesis, growth in *C. gigas*, a trait which has been under selection pressure in the hatchery, seems to be highly dependent on the environment, whereas other traits such as survival in the presence of disease seem dependent on the family (Dégremont *et al.* 2005; Evans & Langdon 2006). Expression of phenotypes can be maintained within families across a range of environments by epigenetic mechanisms (Gavery & Roberts 2017; Uren Webster *et al.* 2018).

Other omic techniques, such as proteomics and metabolomics, provide a direct measurement of expressed phenotypes, and are therefore valuable tools to explore the genotype-phenotype link and evaluate performance (Laudicella *et al.* 2020). Further studies investigating the relation between specific environmental conditions utilizing a holistic omic approach may allow to understand and control for *GxE* in bivalve breeding programmes and are critical to improve aquaculture (Fig. 2).

Implications on selective breeding under a changing climate

Shifts in sea surface salinity, temperature and ocean chemistry (e.g. ocean acidification), alterations in precipitation patterns as well as stronger and more frequent heat waves, are some of the main consequences of climate change to the marine environment predicted for the coming decades (IPCC 2018).

As ectothermic calcifying organisms, marine bivalves are particularly vulnerable to climate change. Shell dissolution and decreased shell growth caused by ocean acidification have been described in marine bivalves (Melzner *et al.* 2011). Higher sea surface temperatures, especially in summer months, may challenge species with lower thermal tolerance (Steeves *et al.* 2018). Fluctuating sea surface salinities may have deleterious implications for shell growth (Riisgård *et al.* 2012) whilst the interactions of this factor with increased temperature or hypercapnia (elevated CO_2) can increase mortality (Rybovich *et al.* 2016) and reduce hardness and resistance of shells (Dickinson *et al.* 2012). Phytoplankton communities are likely to be impacted by climate change (Käse & Geuer 2018), and temporal shifts in species abundance and composition may impact the nutrient uptake in marine bivalves, limiting physiological and biological processes. Climate change may also contribute to lowering the immune response of bivalves (Mackenzie *et al.* 2014), and modify host–pathogen interactions, increasing sensitivity towards diseases (Asplund *et al.* 2014).

The grow-out phase of bivalve aquaculture takes place in the natural environment. Therefore, the implications of climate change are not restricted to wild populations. Strong changes in local environmental conditions may limit production and force the relocation of grow-out sites to suitable areas. Environmental changes and increased disease outbreaks might lead to severe mortality and considerable economic losses in this industry and restrictions in spat commercialization may be needed to avoid the further spread of diseases. Thinner and weaker shells will facilitate their rupture during transportation. Hatchery propagation may also be impaired by climate change to a certain degree, as those rely on natural sea water supply. Therefore, there is an imminent need for research to develop to develop bivalve strains robust to climate change and resilient towards diseases.

Epigenetic processes can contribute to rapid adaptation towards environmental stressors generated by climate change. In *S. glomerata*, short-term exposure to elevated CO_2 concentration not only increases resilience of exposed individuals, but can also be passed through generations (Parker *et al.* 2015). Such resilience has been associated with a change in regulation of genes associated with stress related functions (Goncalves *et al.* 2016). Accordingly. empirical evidence demonstrates that low pH stress (pH 7.4) can modify the methylation patterns of Crassostrea hongkongenssis pediveliger larvae (Lim *et al.* 2021). Genomic processes, in turn, are involved in long term adaptation to environmental changes.

Identifying the mechanisms acting behind *GxE* interactions which increase performance of a species under climate change-associated stressors is an important step to characterize the genomic and epigenomic profile of robust genotypes. Accordingly, *GxE* can be exploited in breeding programmes to increase environmental resilience and the application of genomic selection can fast-track the development of such lines (Mulder 2016). The growing body of high quality assembled genomes facilitate the precise identification of genomic regions linked to traits responsible for environmental resilience. The application of genomic selection, or gene editing approaches, can then facilitate the development of robust lines, or lines able to withstand suboptimal environmental conditions relevant to a certain grow-out region (e.g. elevated temperature, low pH). As hatcheries allow for the control of genetic stocks, indoor propagation is undoubtedly an essential asset to guarantee the development of lines able to thrive under future predicted environmental scenarios.

Summary and future perspectives

A gap in knowledge remains on how domestication selection and husbandry practices can constrain genetic variability of hatchery-propagated stock during early life stages in marine bivalves (Fig. 2). Despite the negative implications of inbreeding load on performance, the control of reproductive output, differential performance of genotypes and genetic variability of stock remains relatively low in bivalve

production. Such lack of control may hinder spat performance and consequently aquaculture production. Future advances in bivalve production and selective breeding require an understanding and optimization of hatchery production processes in order to maximize genetic gain.

Selection pressures acting in hatcheries differ from those acting on the populations in their natural environment. In contrast to wild populations that face fluctuating environmental conditions, farmed stocks are produced under relatively stable, benign, conditions but exposed to other stresses such as elevated densities and handling practices. Organisms reared in hatcheries, naïve to the wild, might lack resilience to environmental variation due to domestication selection and/or epigenetic mechanisms. In addition, negative *GxE* interactions can be detrimental for production and can be compounded by artificial bottlenecking or epigenetic alterations caused by the hatchery environment. However, it is still not fully understood whether selection for phenotypes that enhance hatchery production contribute to adult performance during the grow-out phase. Therefore, whilst performance of larval stages must remain as an important component of breeding programmes and hatchery production, it is key to consider traits related to the challenges these larvae will face during the grow-out and production phases.

Genomic resources will contribute with the understanding of evolutionary and adaptive processes, as well as those which are linked to domestication (Yáñez *et al.* 2015). Elucidating the (epi)genomic mechanisms which underpin the expressed phenotypes will allow the divergence of selection from classic commercial traits towards broad environmental resilience (either outperforming or generalist genotypes). Integrating robustness as a founding criterion for selection can potentially contribute to increase grow-out productivity, especially in light of climate change.

Genomic selection can favour the development of genetically improved lines for multiple traits, facilitate the management of genetic variability (D'Ambrosio *et al.* 2019) and potentially reduce environmental sensitivity accounting for GxE (Mulder 2016). Most importantly, the implementation of such selection approaches is key to the sustainable optimization of bivalve aquaculture production, particularly in the light of climate change. It is crucial to focus resources on developing environmentally robust lines. However, progress of marker assisted and genomic selection in bivalve aquaculture will require a greater control of hatchery practices to allow sources of unaccounted genetic variation to be minimized and genetic gain to be maximized.

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